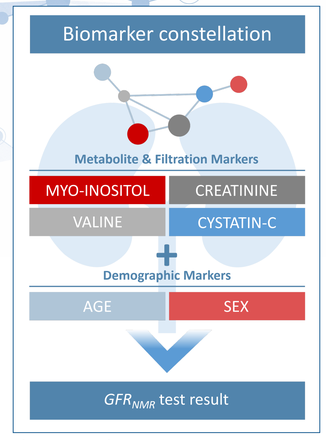
Analytical Validation of GFR_{NMR}: A Blood-Based Multiple Biomarker Assay for Accurate Estimation of Glomerular Filtration Rate

Markus Fuhrmann[1], Amauri Schwaeble Santamaria[1], Jeffrey W Meeusen[2], Marianna Fernandes[3], Frank Stämmler[1], Eric Schiffer[1]

[1] Department of Research and Development, numares AG, 93053 Regensburg, Germany, [2] Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA, [3] Boston Heart Diagnostics, Framingham, MA, USA

BACKGROUND



Accurate and precise monitoring of kidney function is critical for a timely and reliable diagnosis of chronic kidney disease (CKD). Determination of kidney function usually involves the estimation of the glomerular filtration rate (eGFR). We recently reported the clinical performance of a new eGFR test* (GFR_{NMR}) using nuclear magnetic resonance (NMR) measurement of serum myo-inositol, valine, and creatinine, in combination with serum cystatin C, age and sex. GFR_{NMR} had a lower bias to tracer measured GFR (mGFR) than existing eGFR equations, with a median bias (95% confidence interval [CI]) of 0.0 (1.0; 1.0) ml/min/1.73m² vs. -6.0 (-7.0; -5.0) ml/min/1.73m² for the CKD Epidemiology Collaboration equation that combines creatinine and cystatin C (CKD-EPI_{2012}) ($p < 0.0001$). Accuracy (95% CI) within 15% of mGFR (1-P15) was 38.8% (34.3; 42.5) for GFR_{NMR} vs. 47.3% (43.2; 51.5) for CKD-EPI_{2012} ($p < 0.010$). We here describe the analytical performance evaluation of GFR_{NMR} according to Clinical and Laboratory Standards Institute (CLSI) guidelines.

METHODS

Single-donor human serum was collected and used for the measurement of cystatin C on a Tina-Quant Cystatin C Gen.2 assay (Roche) and NMR samples, prepared by mixing 540 µL serum with 60 µL additives solution, were measured without pre-separation on a Bruker Avance III 600 MHz. All assessments and calculations were performed according to CLSI guidance. Limit of blank, limit of detection, and limit of quantification were determined for creatinine, valine and myo-inositol, as well as linearity and trueness. Single-site and multi-site precisions, i.e., repeatability, between-run precision, between-day precision and within-laboratory precision, were determined for the creatinine, valine and myo-inositol analyte measurements and the GFR_{NMR} equation. Sample stability experiments were conducted for storage at 2–10°C and on-board the sample changer at 6°C. Interference testing was performed for 40 substances as an interference screen followed by a dose-response experiment, if applicable.

RESULTS

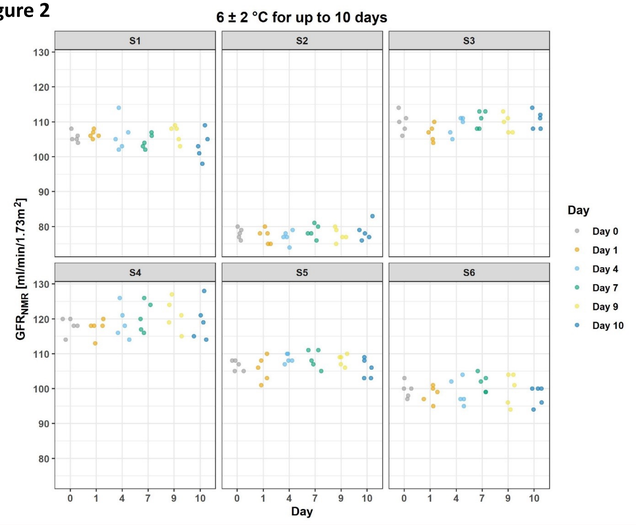
Single-site, within-laboratory coefficients of variation (CVs) of the GFR_{NMR} equation from 4 serum pools and 480 measurements did not exceed 4.3%, with a maximum repeatability CV of 3.7% (Fig. 1). Three-site reproducibility from 4 serum pools and 360 measurements demonstrated a CV of 5.9% (Fig. 1). Serum GFR_{NMR} stability of 8 days at 2–10°C and on board for 10 days at 6 °C (Fig. 2) was demonstrated with a non-significant linear regression slope ($p > 0.05$).

Figure 1

Precision	N	Pool	Mean [mL/min/1.73 m²]	Repeatability [CV%]	Within-Laboratory [CV%]	Reproducibility [CV%]
Single-site [1]	480	P1	53.5	2.8	3.9	n.a.
		P2	55.4	2.9	3.4	n.a.
		P3	78.1	3.7	3.9	n.a.
		P4	82.4	3.6	4.3	n.a.
Multi-site [2]	360	P1	55.4	3.3	3.3	4.3
		P2	84.5	5.1	5.8	5.9
		P3	84.7	2.9	4.4	4.4
		P4	86.6	2.8	3.5	4.0

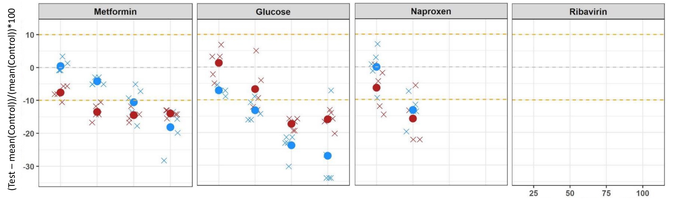
[1] 1 site x 4 serum pools x 3 replicates / pool x 2 runs / day x 20 days (n = 480); [2] 3 sites x 4 serum pools x 6 replicates / pool x 1 run / day x 5 days (n = 360).

Figure 2



4/40 interfering substances resulted in underestimated GFR_{NMR} (for glucose and metformin) or a loss of results (for naproxen and ribavirin) in concentrations twice as high as usual clinical doses (Fig. 3).

Figure 3



Dose response interference experiments. Strip plots showing relative biases of GFR_{NMR} in serum pools spiked with increasing concentrations (0%, 25%, 50%, 75% and 100%) up to three times therapeutic daily dose of the indicated substances. Colors indicate pools with lower (blue) or higher (red) GFR.

CONCLUSIONS

Prior study data have shown superior clinical performance of the GFR_{NMR} test in accurately detecting kidney function compared to existing eGFR equations. We demonstrated excellent analytical performance of the novel GFR_{NMR} test including compatible CVs, sample stability consistent with clinical settings, and no clinically relevant interferences from substances. Pre-separation for individual biomarker measurements was not required. Implementation of GFR_{NMR} on a fully automated, standardized NMR platform is compatible with routine clinical practice in a range of laboratory settings.

*Available as a CE-labeled in vitro diagnostic product in the European Union and as Research-Use-Only product in the U.S. numares´ products have not yet been approved or cleared by the U.S. Food and Drug Administration.

Poster presented, AACC Annual Scientific Meeting 2022, Chicago, IL., # B-300.

Follow numares: @numares AG @numares AG @Agnumares numares NMR diagnostics

Eric.Schiffer@numares.com www.numares.com